Instructions for Use

SALSA® MLPA® Probemix P043 APC

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD022, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P043 APC product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P043 APC**
Version	E1
Catalogue numbers	P043-025R (25 reactions) P043-050R (50 reactions) P043-100R (100 reactions)
Basic UDI-DI	872021148P0435N
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD022	SD022

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL COSTA RICA
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version D1, four new MUTYH probes and two GREM1 upstream probes were included. One MUTYH probe and three reference probes were removed.

1. Intended Purpose

The SALSA MLPA Probemix P043 APC is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions and duplications in the *APC* gene in order to confirm a potential cause for and clinical diagnosis of familial adenomatous polyposis (FAP). Additionally, P043 APC is intended for the detection of deletions in the *MUTYH* gene in order to confirm a potential cause for and clinical diagnosis of *MUTYH*-associated polyposis (MAP). This probemix can also detect the presence of the two most common point mutations in the *MUTYH* gene among people from European descent: c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp). Moreover, P043 APC is intended for the detection of duplications in the upstream region of the *GREM1* gene in order to confirm a potential cause for and clinical diagnosis of hereditary mixed polyposis syndrome (HMPS1). P043 APC is also intended for molecular genetic testing of at-risk family members. The probemix is to be used with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P043 APC should be confirmed with a different technique. In particular, CNVs detected by only a single probe as well as the two *MUTYH* point mutations always require confirmation by another method. Most defects in the *APC* and *MUTYH* genes are point mutations, which will not be detected by MLPA, with exception of the two aforementioned *MUTYH* point mutations. It is therefore recommended to use this assay in combination with sequence analysis. FAP has a high incidence of mosaicism and mosaic *APC* mutations may not be detectable in blood. Not all exons of *MUTYH* are covered in this probemix.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes except for mutation-specific probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of hereditary predisposition to cancer.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Binning DNA (Initial Experiment)	• SALSA Binning DNA SD022, provided by MRC Holland • Can be used in initial experiment to determine suitable bin set • Should never be used as a reference sample	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• In the validation experiments of this probemix, the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.	

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*APC*, *MUTYH* and *GREM1*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of Mutation-Specific Probes

Signal Strength	Mutation Status
≥10% median peak height reference probes	Mutation *MUTYH* c.536A>G or *MUTYH* c.1187G>A is detected (expected only in positive samples)
<10% median peak height reference probes	Mutation *MUTYH* c.536A>G or *MUTYH* c.1187G>A is not detected (expected in most samples from healthy individuals)

These final ratios are only valid for germline testing.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy numbers in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P043 APC in 46 samples from healthy individuals with normal copy numbers and 2 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.</td></tr>
<tr><td>Expected values for point mutation detection in normal and affected populations</td><td>The mutation-specific probe will only generate a signal when the MUTYH c.536A>G (p.Tyr179Cys; 188 nt) and/or MUTYH c.1187G>A (p.Gly396Asp; 193 nt) are present. Please note that background signals of the mutation-specific probe can be expected above the threshold in some cases. Users should always compare the relative peak height of the mutation-specific probe in mutation-positive samples to the relative peak height in reference samples. A clear signal (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes.

The presence or absence for the mutation-specific probes was verified with SALSA MLPA Probemix P043 APC using one sample positive for the MUTYH c.536A>G (p.Tyr179Cys) mutation, one sample positive for the MUTYH c.1187G>A (p.Gly396Asp) mutation, and 46 samples from healthy individuals with normal copy numbers. The expected results were found in all tested samples.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P043 APC on four samples with known CNVs/mutations and on one sample without any mutation. Expected results were obtained using both the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using SALSA MLPA Probemix P043 APC was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/mutations. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 517/525 measurements
Mutation: Expected signal for 30/30 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 525/525 measurements
Mutation: Expected signal for 30/30 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 525/525 measurements
Mutation: Expected signal for 30/30 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 525/525 measurements
Mutation: Expected signal for 30/30 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 506/525 measurements
Mutation: Expected signal for 30/30 measurements</td></tr>
</table>
* Results are summarised for all probes across five samples tested in triplicate.

EDTA and hemoglobin had the largest effects on copy number determination: FRs within incorrect or ambiguous ranges were obtained in almost all samples. DNA extraction methods from blood remove hemoglobin, therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. EDTA had a mild effect on copy number determination. Such values would lead to delayed results at most, as the assay may have to be repeated. No false positives or false negatives would ensue.</td></tr>
</table>

	To minimise variability across samples, all samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, seven previously genotyped samples were tested using SALSA MLPA Probemix P043 APC and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutations over multiple days, and by multiple operators. Results showed a correct call in 2768/2775 measurements, leading to a precision of >99%.
Clinical validity*	*APC*: large deletions or duplications in the *APC* gene explain 8-12% of the (attenuated) familial adenomatous polyposis cases (GeneReviews). *MUTYH*: less than 1% of the *MUTYH*-associated polyposis cases are caused by large deletions in the *MUTYH* gene (GeneReviews). Approximately 70% of patients with *MUTYH*-associated polyposis harbours at least one of the *MUTYH* c.536A>G (p.Tyr179Cys) or *MUTYH* c.1187G>A (p.Gly396Asp) variants (GeneReviews). *GREM1*: 2.4% of colorectal polyposis cases are caused by large duplications in the upstream region of the *GREM1* gene, leading to a clinical diagnosis of hereditary mixed polyposis syndrome (Yang et al. 2025) *(Based on a 2004-2025 literature review)

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Mutation	Length (nt)	Probe number	Warnings
1p34.1	*MUTYH*	Exon 16	2.2 kb		148	21271-L29806	
1p34.1	*MUTYH*	Exon 13	0.6 kb	c.1187G>A (G396D)	193	21267-SP0655-L23442	§ Ж
1p34.1	*MUTYH*	Exon 11	0.7 kb		136	21270-L28956	
1p34.1	*MUTYH*	Exon 7	1.6 kb	c.536A>G (Y179C)	188	18416-SP0654-L29811	§ Ж
1p34.1	*MUTYH*	Exon 2	5.9 kb		346	14912-L19331	
1p34.1	*MUTYH*	Exon 1			253	02110-L04024	
5q22.2	*APC*	Upstream (1)	0.4 kb		260	15274-L19324	∑
5q22.2	*APC*	Upstream (1)	29.7 kb		274	15276-L19326	Δ ∑
5q22.2	*APC*	Upstream (2)	0.3 kb		142	01532-L01743	∑
5q22.2	*APC*	Exon 1 (2)	0.5 kb		154	11988-L29807	+ ∑
5q22.2	*APC*	Intron 1 (3)	16.5 kb		172	01534-L29810	Ø
5q22.2	*APC*	Exon 2 (4)	11.5 kb		178	01535-L19321	
5q22.2	*APC*	Exon 3 (5)	0.9 kb		202	01536-L29812	
5q22.2	*APC*	Exon 4 (6)	8.4 kb		209	01537-L29813	
5q22.2	*APC*	Exon 5 (7)	5.2 kb		224	01538-L29814	
5q22.2	*APC*	Exon 6 (8)	11.6 kb		229	01539-L01337	
5q22.2	*APC*	Exon 7 (9)	8.8 kb		246	01540-L00983	
5q22.2	*APC*	Exon 8 (10)	14.2 kb		267	01541-L19325	
5q22.2	*APC*	Exon 9 (11)	3.8 kb		282	01542-L19327	
5q22.2	*APC*	Exon 10 (12)	2.6 kb		289	11990-L19328	
5q22.2	*APC*	Exon 11 (13)	1.4 kb		306	01544-L00987	
5q22.2	*APC*	Intron 11 (Intron 13)	3.8 kb		409	03323-L02526	Ø
5q22.2	*APC*	Exon 12 (14)	0.8 kb		312	01545-L01338	
5q22.2	*APC*	Exon 13 (15)	0.9 kb		328	01774-L01340	
5q22.2	*APC*	Exon 14 (16)	6.2 kb		337	01700-L01341	
5q22.2	*APC*	Exon 15 (17)	2.7 kb		365	01548-L20445	
5q22.2	*APC*	Exon 16 (18)	1.0 kb		382	21268-L29625	
5q22.2	*APC*	Exon 16 (18)	0.7 kb	c.3183_3187delACAAA (wild type)	419	03324-L29817	∞
5q22.2	*APC*	Exon 16 (18)	0.9 kb	c.3927_3931delAAAGA (wild type)	427	03325-L02528	∞
5q22.2	*APC*	Exon 16 (18)	0.7 kb		357	21269-L29815	
5q22.2	*APC*	Exon 16 (18)	0.7 kb		454	18175-L22760	
5q22.2	*APC*	Exon 16 (18)	0.7 kb		166	18176-L29809	
5q22.2	*APC*	Exon 16 (18)	0.7 kb		463	18177-L22762	
5q22.2	*APC*	Exon 16 (18)	0.8 kb		436	18178-L24041	
5q22.2	*APC*	Exon 16 (18)			391	12035-L29816	
15q13.3	*GREM1*	Upstream	8.4 kb		217	21272-L23310	∫
15q13.3	*GREM1*	Upstream			474	21273-L23308	
1p	Reference				400	08871-L08927	
4q	Reference				483	12761-L29818	
5q	Reference				130	00797-L00463	
6q	Reference				445	17736-L21863	
7q	Reference				297	04355-L19329	
8q	Reference				238	10089-L10513	
16q	Reference				373	15525-L17380	
17p	Reference				160	13447-L29808	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *APC* exon numbers are derived from MANE project and are based on MANE Select transcript. For *MUTYH*, exon numbers are based on the MANE Plus Clinical transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region, are altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

§	These probes will only generate a signal when the mutation is present. Masking of the probe signal can occur if another mutation or SNV is present **on the same allele.**
∞	Wild type sequence detected. A lowered probe signal can be due to the presence of the APC c.3183_3187delACAAA (rs587779352; p.Lys1061_Gln1062insTer; codon 1061) mutation for the 419 nt probe or the presence of the APC c.3927_3931delAAAGA (rs121913224; p.Glu1309fs; codon 1309) mutation for the 427 nt probe. Other variants near the ligation site or a deletion can also cause a lowered signal. A positive result must be confirmed by another method.
Δ	This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
Ж	These probes consist of three parts and have two ligation sites. A low signal of these probes can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
Ø	These probes target a sequence outside of the coding region of the MANE Select transcript. Copy number alterations of only (one of) these probes are of unknown clinical significance.
+	The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
∑	Deletions and duplications in the promoter 1A and 1B region of the *APC* gene have been described before and were linked to a hereditary predisposition to adenomatous polyposis (Kadiyska et al. 2014, Loginova et al. 2023, Marabelli et al. 2017). Copy number changes in promoter region 1A of *APC* can be detected by the 142 and 154 nt probes. Copy number changes in promoter region 1B of *APC* can be detected by the 260 and 274 nt probes.
∫	The 16 kb duplication reported in Rohlin et al. 2015 affects the 217 nt probe only. Other duplications in the upstream region of the *GREM1* gene that have been found in other publications affect both the 217 and the 474 nt probes (McKenna et al. 2019, Venkatachalam et al. 2011).

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.
5. A non-specific peak with a length of approximately 239 nt was observed in the no-DNA control. This peak has a height of <25% of the median of the four Q-fragments and is therefore not expected to affect MLPA reactions when sufficient (50-250 ng) sample DNA is used. Deviations from the protocol may increase the height of this peak in reactions with and without DNA.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The two MUTYH mutation-specific probes are only intended to determine the presence (or absence) of the mutation, and are not meant to determine the zygosity of the mutation. Follow-up with another method is advised.
2. Only *MUTYH* exon 1, 2, 11 and 16 are covered in this probemix. For additional coverage, SALSA® MLPA® Probemix P378 MUTYH is available.

Technique-specific limitations

See the MLPA General Protocol.

9. References Cited in this IFU

1. Kadiyska TK et al. (2014). APC promoter 1B deletion in familial polyposis--implications for mutation-negative families. *Clin Genet.* 85:452-457.
2. Loginova AN et al. (2023). Large Rearrangements in Genes Responsible for Familial Adenomatous Polyposis, MUTYH-Associated Polyposis and Peutz–Jeghers Syndrome in Russian Patients.
3. Marabelli M et al. (2017). A novel APC promoter 1B deletion shows a founder effect in Italian patients with classical familial adenomatous polyposis phenotype. *Genes Chromosomes Cancer.* 56:846-854.
4. McKenna DB et al. (2019). Identification of a novel GREM1 duplication in a patient with multiple colon polyps. *Fam Cancer.* 18:63-66.
5. Venkatachalam R et al. (2011). Identification of candidate predisposing copy number variants in familial and early-onset colorectal cancer patients. *Int J Cancer.* 129:1635-1642.
6. Yang M et al. (2025). Uncovering the genetic variation spectrum of colorectal polyposis from a multicentre cohort in China. *npj Precision Oncology.* 9:75.

Implemented changes in the product description
Version E1-10 – 29 September 2025 (03S) - Intended purpose updated, specifying that the assay is manual and that not all exons of the *MUTYH* gene are covered by this assay. CNV type detected in the *MUTYH* gene was limited to deletions. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - SNV rs116020626 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Exon numbering updated for all APC probes. - Warning for target sequences outside of the MANE Select transcript known coding region added for probe 01534-L29810. - Warnings for a ligation site >20 nt away from the nearest exon added for probe 11988-L29807. - Warning for probes targeting *APC* promoter region 1A and 1B adjusted. - Warning ∫ updated for clarification. - Precaution added for non-specific peak around 239 nt in no-DNA control. - Limitation regarding the coverage of *MUTYH* in this probemix added. - Probemix is now IVDR certified.